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PRINCIPAL INVESTIGATOR: Peter J. Kushner, Ph.D.

CONTRACTING ORGANIZATION: University of California,
San Francisco
San Francisco, California 94143-0962

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FOREWORD

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INTRODUCTION

Progestins and glucocorticoids are sometimes used as second line therapy for breast cancer that has become resistant to antiestrogens. Estrogen action is opposed by glucocorticoids and by progestins in several physiologic and pathophysiologic processes. For example, estrogen stimulates uterine growth and DNA synthesis. Glucocorticoids and progestins block these uterotrophic effects (1). In the stress response, estrogen treatment is associated with increased levels of circulating corticosterone (2), whereas glucocorticoids down-regulate hypothalamic-pituitary-adrenal axis activation to reduce circulating glucocorticoid levels. Estrogen treatment is also associated with lesion-induced neuronal sprouting *in vivo* (3) and neurite outgrowth in culture (4). Conversely, glucocorticoids in excess are associated with dendritic atrophy and cell death in pyramidal neurons of the hippocampus (5). In bone, estrogen blocks osteoclast development and activity (6); in its absence, osteoclast activity increases leading to osteopenia (7). Conversely, the glucocorticoid agonist dexamethasone (Dex) induces osteoclast formation (8). In breast cell lines, estrogen promotes growth while glucocorticoids inhibit it (9). Given the frequency of these opposing effects, we sought to elucidate a mechanism by which estrogen and glucocorticoid actions might be integrated.

Steroids act by binding to cognate receptors. The steroid-receptor complex then binds DNA at a hormone response element and activates gene transcription. In order for estrogens and glucocorticoids to counteract each other at the level of transcription, a given cell would have to express both receptors (ER and GR). In the uterus there is evidence that ER and GR co-exist in the endometrium (10). In the brain, maps of ER and GR immunoreactivity and mRNA localization suggest co-localization in certain cerebral nuclei, such as the paraventricular nucleus of the hypothalamus, the hypothalamic arcuate nucleus, and the central nucleus of the amygdala (11, 12). In bone, ER and GR have been found in cultured osteoblast-like cells (13). ER has also been

demonstrated in osteoclasts (6) and data suggest that Dex regulates metabolism in these cells (14), raising the possibility that osteoclasts contain functional GR as well. Lastly, numerous breast tumor cell lines have been demonstrated to have both ER and GR (15). Therefore, there is potential for ER/GR interactions at the level of transcription in numerous cell lines and tissue types.

The mechanism by which the ER and GR interact at the level of transcription must involve a process distinct from steroid receptor/hormone response element interactions. These interactions are highly specific as dictated by differences in the DNA binding regions of the estrogen and glucocorticoid receptors and in the sequence specificity of their cognate response elements (16, 17). An alternate explanation could involve interactions between steroid receptors and other transcription factors. The ER, GR, and other nuclear hormone receptors have been shown to alter transcription through the AP-1 response element which is bound by the transcription factors Jun and Fos. In fact, estrogens and glucocorticoids have opposing effects at this response element: estrogens stimulate AP-1 activated transcription (18, 19) while glucocorticoids inhibit it (20). Therefore, it might be possible for estrogens and glucocorticoids to influence each others ability to modulate transcription through the AP-1 site.

Given these common tissue targets, the presence of ER and GR in cell types contained within them, and the large number of genes regulated by members of the AP-1 family, we sought to determine if the AP-1 response element could functionally integrate the transcriptional effects of estrogens and glucocorticoids. We characterized this interaction in cells originally derived from a uterine cervical adenocarcinoma (HeLa) and in a hypothalamic cell line (GT1-1; (21)). We also tested the possibility that ER and progesterone receptor types A and B (PR-A and -B) might interact at the AP-1 response element.

BODY

MATERIALS AND METHODS

Plasmids

Coll73-LUC and Coll60-CAT have been previously described (19, 22). Coll73-LUC consists of -73 to +63 of the collagenase promoter upstream of the Luciferase reporter gene. Coll517-CAT and Coll517mAP-1-CAT each contain -517 to +63 of the collagenase promoter (19).

Coll517mAP-1-CAT contains three point mutations in the consensus AP-1 response element (TGAGTCA mutated to GTACTCA). ColALuc contains the collagenase AP-1 response element upstream of the minimal drosophila alcohol dehydrogenase promoter (23). The ER expression vectors have been previously described: pHE0 (24), pHEG0 (25), HE11 (26), and HE15 (27). pHE0 contains a point mutation (Gly400Val). pHEG0 is the wild type ER. pHE0 has reduced affinity for estrogens which allows for studies in cell culture without inadvertent activation. The protein coding regions of the ER plasmids were cloned into the multiple cloning site of the pSG5 expression vector. pRSVhGR (28) consists of a cDNA encoding the human GR coding region inserted into an expression vector driven by the Rous sarcoma virus promoter. The PR-A (pHPR-60) and PR-B (hPR65) plasmids were derived from T47D cDNA and genomic DNA (29,30) and cloned into an expression vector derived from pLEN (31). They were obtained from G. Greene (A. Robinson and G. Greene, manuscript in preparation). The human c-Jun (32) and rat c-Fos (33), have been previously described. The beta-actin-hCG construct has been previously described (22). The pJ3-*LacZ* plasmid was constructed by Jay Morgenstern. It is pBR322-based and contains an SV40 promoter which activates *LacZ*.

Cells

All cells were maintained in DME medium without phenol red. The medium is supplemented by serum (Sigma) which we test for low estrogenic activity prior to use. Charcoal- and heat- (55°C x

1/2 hr.) treated serum was used in the GT1-1, and in all PR experiments. In these experiments cells were treated with media containing charcoal-treated serum the night prior to transfection.

Transfection

Cells were transfected by electroporation as previously described (19). Briefly, 1-2 million cells from just confluent plates were used for each cuvette. Cells were electroporated at .24 kV in electroporation buffer. The electroporated cells were resuspended in medium which was then divided into six well plates. The efficiency of transfection was monitored by co-transfection with either a β hCG reporter driven by an actin promoter (22) or by co-transfection with pJ3LACZ. CAT or luciferase activity was then corrected by dividing by hCG levels or β -galactosidase activity. Five micrograms of collagenase reporter plasmid, and 1 μ g of GR expression vector were used in all experiments unless otherwise indicated.

Cell Treatments

Cells were treated either immediately or up to 6 hours after transfection. They were then harvested at approximately 40 hrs. after plating. Dexamethasone, estradiol, and R5020 were all used at 10^{-7} M. Tamoxifen was used at 5×10^{-6} M. PMA (Sigma) was suspended in DMSO and cells were treated at 10^{-7} M; TNF- α (R&D Systems, Minneapolis, MN) was resuspended in 0.1% BSA and cells were treated at 10 ng/ml.

CAT, Luciferase, hCG, and β -Galactosidase Assays

CAT, luciferase, and hCG assays were performed as described (19, 22). A commercial luminescent assay (Tropix; Bedford, MA) was used for β -galactosidase measurements.

Data analysis

In most figures data has been expressed relatively to permit statistical analysis of data from separate experiments. The relative number, fold induction or percent stimulation, was averaged from 2-5

experiments as indicated in the figure legends. Standard deviation was calculated for each averaged point except for the reference which was set to one (fold induction) or 100% (percent stimulation). Fold induction was calculated as the ratio of a steroid treatment to the "No Steroid" treatment point. Percent stimulation was calculated as percent of estradiol treatment. In some figures representative data are shown instead of averaged data. This permits evaluation of the effect of a co-transfected plasmid or AP-1 activator treatment on transcription in the absence of steroid treatment. In all cases the data represented has been repeated in ≥ 3 similar experiments.

RESULTS AND DISCUSSION

GR inhibits ER transcriptional activation through the AP-1 response element.

It has been previously demonstrated that estrogens stimulate and glucocorticoids inhibit basal activity of a truncated collagenase promoter which contains the consensus AP-1 response element (Coll73) (18-20). Since both steroids modulate transcriptional activation through the AP-1 response element, we asked whether the ER and GR could influence each other's transcriptional effects at this site.

HeLa cells were transfected with ER (HE0) and the truncated collagenase promoter (Coll73-LUC) (Fig. 1A), then treated with Dex, estradiol, or Dex+ estradiol. As previously reported, Dex inhibited and estradiol stimulated transcription through this promoter. When both steroids were added, GR blocked estradiol-stimulated transcription (Fig. 1B). A similar ER-GR interaction occurs with both HE0 and HEG0, which encodes the wild type receptor (data not shown).

To determine whether or not the AP-1 response element is required for the glucocorticoid/estrogen interaction, the steroid responses were evaluated at a longer form of the collagenase promoter in the presence of an intact or mutated AP-1 response element (Coll517 or Coll517mAP-1, respectively). As was the case with Coll73, Dex blocked estradiol activity through an intact AP-1 response element. The steroid responses were abrogated when the promoter bearing the mutated AP-1 response element was used (Fig. 1C; (19, 20 and references therein)). The minor steroid effects seen in Fig. 1C are not reproducible (data not shown). Steroid effects were also attenuated when HeLa cells were transfected with Coll73-CAT deleted of the AP-1 response element (Coll60-LUC; (19, 20) and data not shown). Dex, then, is able to block estradiol stimulation of transcriptional activation mediated by the AP-1 response element.

ER and GR functionally compete at the AP-1 response element.

The above finding that Dex could block estradiol stimulation of transcriptional activity at the AP-1 site suggested that the ER and GR might functionally compete at this response element. We sought to determine whether or not this was the case. We transfected HeLa cells with increasing amounts of ER in the presence of a constant amount of co-transfected GR (1 μ g). At high levels of transfected ER, Dex was unable to inhibit the estradiol response (Fig. 2A&B). We then transfected increasing amounts of GR in the presence of a constant, high level of co-transfected ER. In the presence of endogenous levels of GR, Dex was unable to inhibit estradiol stimulation. Dex inhibition was restored by co-transfecting $\geq 1\mu$ g of GR and became more pronounced at higher levels of GR (Fig. 2C&D and data not shown). Taken together, these data and the data presented in Fig. 1 indicate that ER and GR transcriptional actions functionally compete through the AP-1 response element. The competitive nature of this interaction predicts that the net outcome of estrogen and glucocorticoid transcriptional activity at the AP-1 response element will depend on the ratio of ER to GR in a given cell. High levels of ER would result in stimulation and high levels

of GR would result in inhibition. Intermediate levels of each would result in an intermediate effect. In some cases a given proportion of ER:GR might result in the cancellation of any estrogen or glucocorticoid effects at all.

Dexamethasone inhibits estradiol- and tamoxifen- mediated ER activation through the AP-1 response element.

We have proposed that ER stimulation of transcription through the AP-1 response element occurs through more than one pathway (19). The alpha pathway is characterized by tamoxifen-induced transcriptional activation and a requirement for the ER DNA binding domain. Dex inhibited tamoxifen activation (Fig. 3A). As seen with estradiol, the degree of Dex inhibition diminished in the presence of high levels of co-transfected ER (Fig. 3A). A C-terminally deleted ER (HE15) serves as a model of tamoxifen activation. It lacks the activation function in the C-terminus and activates transcription through the activation function in the N-terminal domain. Therefore, it is constitutively active at Coll73 (19). When we transfected cells with increasing amounts of HE15 and treated with vehicle or Dex, Dex inhibited the constitutive activity of HE15 (Fig. 3B). Again, the interaction was functionally competitive; over-expression of HE15 (Fig. 3B) overcame Dex mediated inhibition.

The beta pathway is characterized by estradiol activation and the lack of a requirement for the ER DNA binding domain. To determine whether or not Dex could inhibit estradiol-liganded HE11 (which lacks the DNA binding domain), cells were treated with estradiol, Dex, or Dex+ estradiol, as above. Dex inhibited estradiol activation through HE11 (Fig. 4). Since Dex inhibited tamoxifen stimulation, the constitutive activity of the ER deleted of its C-terminal domain, and estradiol-activated ER deleted of its DNA binding domain (Figs. 3&4), we infer that glucocorticoids can inhibit both alpha and beta pathways of ER stimulation.

c-Jun and c-Fos differentially alter estradiol and Dex effects

It has been demonstrated that individual members of the AP-1 family differentially change the pattern of steroid receptor activation at a hormone response element. For example, increasing amounts of c-Jun and c-Fos progressively attenuate ER activation at an estrogen response element (ERE) in MCF-7 cells whereas transfected JunD does not (34). In addition, the ratio of Jun:Fos in a given cell will change the steroid response to Dex at the AP-1 site (35) and the proliferin composite (GRE/AP-1) response element (36).

We evaluated steroid responses in the presence of increasing amounts of transfected c-Jun or c-Fos expression vectors. As previously demonstrated, c-Jun increased estradiol transcriptional activation at Coll73 (Fig. 5A, (19)). At levels of co-transfected c-Jun which resulted in slightly increased AP-1 activated transcription, estradiol stimulation was potentiated. At levels of co-transfected c-Jun which resulted in marked stimulation of AP-1 activated transcription, further estradiol stimulation of AP-1 activation was no longer present. Dex treatment alone restricted transcriptional activity to low levels at all amounts of transfected c-Jun. In the presence of both Dex and estradiol, the levels of transcription were close to those seen when cells were treated with Dex alone. Co-transfected c-Fos potentiated c-Jun stimulation of estradiol-mediated transcriptional activation (Fig. 5B; (19)). In distinction to transfection with c-Jun alone, transfection with c-Fos alone failed to alter steroid responses. Co-transfection of Jun B and D (0.1 - 3.0 μ g) had minimal effects on the pattern of steroid responses (data not shown). Therefore, individual AP-1 family members appear to have different effects on the profile of steroid responses at the AP-1 site.

Activators of c-Jun differentially alter estradiol and Dex patterns of response at the AP-1 response element.

The phorbol ester PMA, and the cytokine tumor necrosis factor- α (TNF- α) both activate c-Jun. However, they do so through different pathways which ultimately target different c-Jun phosphorylation sites (37, 38). To determine whether or not glucocorticoid and/or estrogen effects at the AP-1 response element would be altered in the presence of these activators, HeLa cells were treated with estradiol and/or Dex in the presence or absence of either PMA (10^{-7} M) or TNF- α (10 ng/ml). These doses resulted in maximal AP-1 activation for each agent (data not shown). PMA treatment in the absence of steroids resulted in a ten fold stimulation of transcriptional activity (Fig 6A, note difference in the scale of the No PMA and PMA axes). The pattern of steroid effects was maintained in the presence of PMA (Fig. 6A). In distinction, estradiol stimulation was no longer apparent in the presence of TNF- α although Dex inhibition was maintained (Fig. 6B). The loss of estradiol stimulation was not a result of altering the functional activity of ER. Cells simultaneously transfected with both Coll73-LUC and ERE-Coll60-CAT failed to show diminished activity of ER at an ERE (data not shown). Therefore, while both of these agents activate c-Jun, they each have different effects on estradiol responses at the AP-1 response element.

The GR inhibits ER stimulation in a hypothalamic cell line.

To determine whether or not the ER/GR/AP-1 response element interaction was restricted to HeLa cells, we repeated our initial experiments in a hypothalamic cell line. GT1-1 cells were derived from a transgenic mouse whose GnRH neurons were targeted for transformation by the SV40 T antigen (21). They express neuronal but not glial markers (21), GnRH (21), and the glucocorticoid receptor (39). We transfected GT1-1 cells with the reporter plasmid ColALuc (23),

ER, and GR. In the absence of co-transfected c-Jun, we failed to observe estradiol stimulation or Dex inhibition of either basal or estradiol stimulated transcription (data not shown). In the presence of transfected c-Jun, the pattern of steroid responses was similar to that seen in HeLa cells (Fig. 1A): estradiol stimulated, and Dex inhibited both basal and estradiol stimulated transcription (Fig. 7). Like HeLa cells (Fig. 1B), GT1-1 cells transfected with a collagenase reporter bearing a mutated AP-1 response element (Coll517mAP-1) failed to show steroid responses when compared to Coll517 (data not shown). These data suggest that in the appropriate state of c-Jun expression, ER and GR may competitively interact to modulate expression of genes activated through the AP-1 response element in neurons (see Discussion).

The progesterone receptor (PR) interacts with ER at the AP-1 site.

Like the glucocorticoids, progestins oppose estrogen actions. Since it has been demonstrated that the PR inhibits PMA activated transcription through the AP-1 response element (40), we asked whether or not the PR could also interact with the ER at the AP-1 response element. In HeLa cells, the progesterone agonist R5020 inhibited the basal activity of an AP-1 site through both PR- A and -B (Fig. 8A). As before, estradiol treatment stimulated transcription. Treatment with both steroids resulted in a loss of RU5020 inhibition. PR-A behavior was then evaluated in a different cell line. In the presence of transfected PR-A, R5020 inhibited the estradiol response in CV-1 cells monkey kidney cells which lack endogenous GR (Fig. 8B). Further, co-transfection of increasing amounts of c-Jun resulted in a pattern similar to that seen in HeLa cells (compare Fig. 5A to 8B): transfecting increased amounts of c-Jun lead to increased estradiol stimulation while RU5020 inhibition of estradiol was maintained at low levels. As in the case of the GR, PR-A responses were not seen in the presence of the collagenase reporter bearing the mutated AP-1 site (Coll517 vs. Coll517mAP-1, data not shown) These data indicate that, like ER and GR, ER and PR influence each other's transcriptional activation properties at the AP-1 response element.

RECOMMENDATIONS IN RELATION TO THE STATEMENT OF WORK.

This year's report is directed to Task 5.- Examination of the AP-1 mediated estrogen and antiestrogen transcriptional response by retinoic acid and its receptor. The motivation for this task was that several nuclear receptors including the thyroid, retinoic acid, glucocorticoid and progesterone receptors were known to have opposite effects to those of the estrogen receptor on genes regulated by AP-1 sites and it was natural to ask what effect they would have on the estrogen and tamoxifen activation of these genes. We decided to slightly modify this aim and to concentrate on the glucocorticoid and progesterone mediated action at AP-1 rather than the retinoid because more was known about the action of the glucocorticoid receptor at AP-1 and because glucocorticoids and progestins are sometimes used as second line therapy after resistance to tamoxifen has developed in the treatment of breast cancer.

CONCLUSIONS

We have demonstrated that the ER functionally interacts with the GR and PR at the consensus AP-1 response element. To characterize the nature of the ER/GR interaction we showed that the interaction is functionally competitive, that Dex inhibits more than one ER ligand and receptor form, and that Dex inhibits ER activation potentiated by co-transfected c-Jun. Further, we found that neither ER/GR nor ER/PR-A interactions were limited to HeLa cells.

The data presented here support the hypothesis that opposing effects of estrogens and glucocorticoids or progestins can be mediated at the level of transcription. It has been previously reported that the ER, GR and PR compete for unidentified factors involved in transcriptional regulation at hormone response elements (41). Here we show that estrogen and glucocorticoid or progesterone receptors influence each other's activity at an element through which they individually regulate transcription: the AP-1 site. This does not preclude that steroid interactions occur through other mechanisms, some of which may include other nuclear transcription factors.

The potential implications of these results are several fold. First, while a cell may be capable of mounting an estrogen or glucocorticoid response at the AP-1 response element, whether or not the response will actually occur will depend on the relative levels of each receptor. Estrogen stimulation of AP-1 regulated genes may be blunted in the presence of glucocorticoids. Conversely, glucocorticoid inhibition could be overcome by estrogen activation. Second, the steroid response will be modulated by the levels and composition of the AP-1 protein complex in the cell. Transfected c-Jun and c-Fos differentially altered the estrogen and glucocorticoid pattern of transcription. Lastly, the steroid responses will also be modified by the activation state of the cell. Certain activators of AP-1 may modulate a steroid response, e.g. TNF- α modulation of estrogen stimulation, while others may not.

There are several candidate genes for which such an ER/GR/ or ER/PR/ AP-1 response element interaction might be important. In the uterus, estradiol treatment increases the level of IGF-1 mRNA and the increase is attenuated by prior administration of Dex (42). Our data from HeLa cells, a cell line originally derived from a uterine cervical adenocarcinoma, may suggest that genes expressed in the uterus have the cellular machinery to integrate ER and GR or PR responses through the AP-1 response element. The ER/PR interaction would be particularly important to pursue in uterine tissues given the number of physiologic estrogen/progestin interactions in that organ. For example, the high estrogen levels of the menstrual follicular phase are associated with proliferation of the endometrial epithelium. The transition from the proliferative to the secretory phase is associated with increased levels of progesterone. It is possible that genes associated with this transition could be jointly regulated by estrogen and progesterone at an AP-1 site.

In the nervous system, estrogens and glucocorticoids regulate the synthesis of numerous neuropeptides including VP, POMC and GnRH (43-46). Since we have shown that the ER/GR/AP-1 response element interaction is present in a hypothalamic cell line, it is possible that neurons which express these genes could have the cellular machinery to integrate estrogen and glucocorticoid or progestin effects at AP-1 sites. In particular, GT1 cells synthesize GnRH and we and others have evidence that they contain functional endogenous ER (data not shown; (47)). Further, GT1 cells contain endogenous GR, which apparently functions to down-regulate GnRH transcription in GT1 cell lines in response to Dex (39). We suggest that GnRH, which contains an AP-1 response element in its promoter (46), could be regulated by estrogens and glucocorticoids in this manner.

The data presented here demonstrate that the AP-1 response element integrates the transcriptional properties of the ER with three other members of the nuclear receptor transcription factor family, the GR and PR-A and PR-B. Multiple receptors in this family have been shown to

act at an AP-1 site (22, 48-51). Therefore, the potential exists for the AP-1 response element to integrate the effect of the ER with other members of the family, as well as to integrate the effects of other superfamily members with each other. Such integration might occur at Jun/Jun, Jun/Fos AP-1 complexes or through shared co-activators. For example, the CREB-Binding Protein (CBP) is a co-activator for AP-1 (52). In turn, CBP has been shown to interact with several members of the steroid receptor superfamily as well as with members of the steroid receptor co-activator (SRC) family (53). Therefore, the functional interaction of the steroid receptors described at the AP-1 site could be mediated not only through AP-1 protein complexes but also through a number of co-activator proteins involved in transducing steroid receptor signals to the basal transcriptional machinery.

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APPENDICES:

FIGURE LEGENDS

Fig. 1. Estradiol and the glucocorticoid dexamethasone modulate each others transcriptional properties at the AP-1 response element.

(A) Structure of collagenase reporter and steroid receptor vectors used. (B) HeLa cells were co-transfected with the Coll73-LUC reporter gene (5 μ g) and the human ER expression vector, pHE0 (5 μ g). After plating they were treated with vehicle, Dex, estradiol, or Dex+Estradiol (10^{-7} M, each steroid) for approximately 40 hrs. then assayed for luciferase activity. The data are from three experiments. Columns represent the average fold induction, defined as the steroid treatment divided by the No Steroid treatment. (C) Three point mutations in the AP-1 site of the collagenase promoter markedly attenuated steroid effects on transcriptional activation. HeLa cells were transfected with 5 μ g of either the intact (Coll517-CAT) or mutated (Coll517mAP-1-CAT) collagenase reporter genes along with GR (1 μ g) and ER (3 μ g) expression vectors. Data are from two experiments. Columns represent the average. (B&C) Error bars represent the standard deviation.

Fig. 2. ER and GR compete at the AP-1 site.

HeLa cells were transfected with the Coll73-LUC reporter gene (5 μ g) and the expression vectors illustrated in Fig. 1A as follows: (A) GR (1 μ g) and increasing amounts of ER as indicated. Columns represent an average of three treatment points from one experiment. (B) Columns represent the average of three experiments expressed as fold induction. (C) Cells were transfected with ER (10 μ g) and increasing amounts of GR. Columns represent the average of three treatment points. (D) Columns represent the average of two experiments not including the experiment shown in C. (A-D) Error bars represent standard deviation. (RLU) Relative light units.

Fig. 3. Dex inhibits both Tamoxifen stimulation and the constitutive activity of the ER deleted of the ligand binding domain (HE15).

HeLa cells were transfected with the Coll73-LUC reporter gene as in Fig. 1. (A&B) Cells were transfected with GR (1 μ g) and increasing amounts of HE0. They were treated with vehicle, Dex, tamoxifen (5×10^{-6} M), or Dex+tamoxifen. (A) Columns represent the average of three treatment points. (B) Cells were transfected with increasing amounts of HE15 and treated with vehicle or Dex. As a control, one set of cells was transfected with HE0 and treated with No Steroid, Dex, estradiol and Dex+estradiol. Columns represent the average of three treatment points. (A&B) Experiments were repeated ≥ 3 . Error bars represent standard deviation.

Fig. 4. Dex inhibits the ER deleted of its DNA binding domain.

HeLa cells were transfected with the Coll73-LUC reporter gene as in Fig. 1 and expression vectors as follows: Empty expression vector (pSG5; 5 μ g), ER (HE0; 5 μ g), and the ER deleted of its DNA binding domain (HE11; 3 and 5 μ g). Cells were treated with steroids as in Fig. 1. Columns represent an average of three treatment points, error bars the standard deviation. The data are representative of similar experiments performed ≥ 3 times.

Fig. 5. Co-transfected c-Jun potentiates steroid effects; co-transfected c-Fos further potentiates c-Jun effects on estradiol stimulation.

HeLa cells were transfected with the Coll73-LUC reporter gene and treated with steroids as in Fig. 1. Cells were co-transfected with ER and GR expression vectors (1 μ g each) and increasing amounts of c-Jun (A) or c-Fos (B). All columns and error bars represent the average of three treatment points except in (B) in which the c-Jun and c-Jun+c-Fos data represent one transfection with one treatment point each. Error bars represent standard deviation. The data are representative of similar experiments performed ≥ 3 times.

Fig. 6. PMA and TNF- α differentially alter steroid responses at Coll73.

HeLa cells were transfected with the Coll73-LUC reporter gene as in Fig. 1. They were co-transfected with ER (5 μ g) and GR (1 μ g) and treated with steroids as in Fig. 1 in the presence or absence of PMA (A) or TNF- α (B) at the doses indicated. (A) *Note* : the scale for *PMA* treated cells is 10x that of cells not treated with PMA ("No PMA"). Columns represent an average of three treatment points, error bars represent standard deviation. The data are representative of similar experiments performed ≥ 3 times. (B) Columns represent an average of three experiments. (A&B) error bars represent standard deviation.

Fig. 7. Dex inhibits estradiol stimulation of transcription through the AP-1 response element in a hypothalamic cell line.

GT1-1 cells were transfected with ColALuc (5 μ g), HE0 (5 μ g), GR (1 μ g), and c-Jun (3 μ g). Cells were treated with steroids 4 hrs. after transfection and harvested 36 hrs. later. The data are expressed as per cent estradiol stimulation. Columns represent the average of three experiments. Error bars represent the standard deviation.

Fig. 8. Estradiol and the progestin RU5020 modulate each others transcriptional properties at the AP-1 response element.

(A) HeLa cells were transfected with ColALuc (5 μ g), ER (1 μ g), PR-A or PR-B (1 μ g), and c-Jun (3 μ g). Data are from four separate transfections from three experiments for PR-A and from two transfections from two experiments for PR-B. Columns represent the average fold induction. (B) CV-1 cells were transfected with ColALuc (5 μ g), ER (HE0, 0.5 μ g), PR-A (1 μ g) and c-Jun (3 μ g). Data is from one experiment. Columns represent the average of two treatment points. Similar experiments have been repeated ≥ 3 times. (A&B) Cells were treated with steroids immediately after transfection and harvested 40 hrs. later. Error bars represent standard deviation.

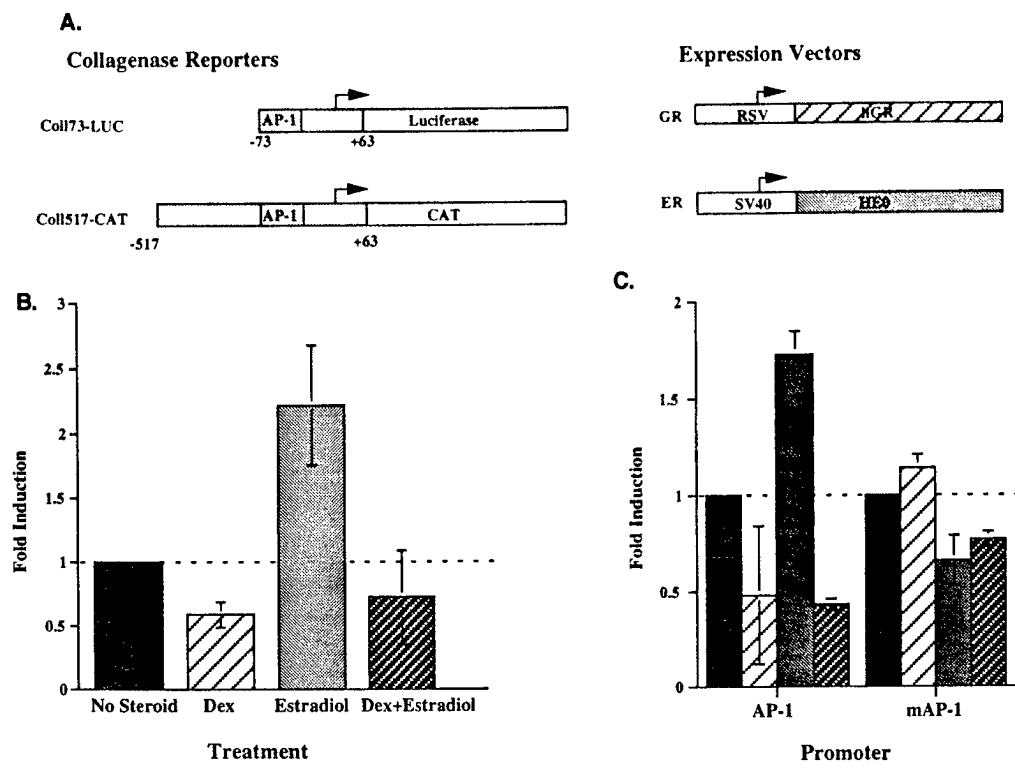


Figure 1

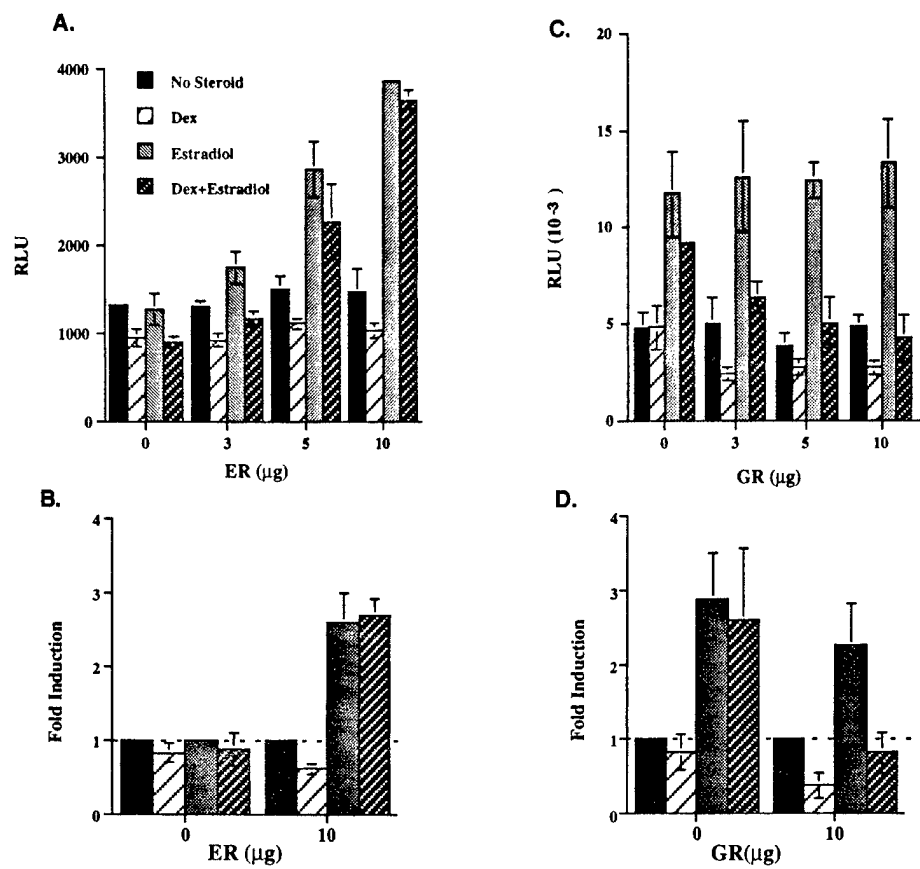


Figure 2

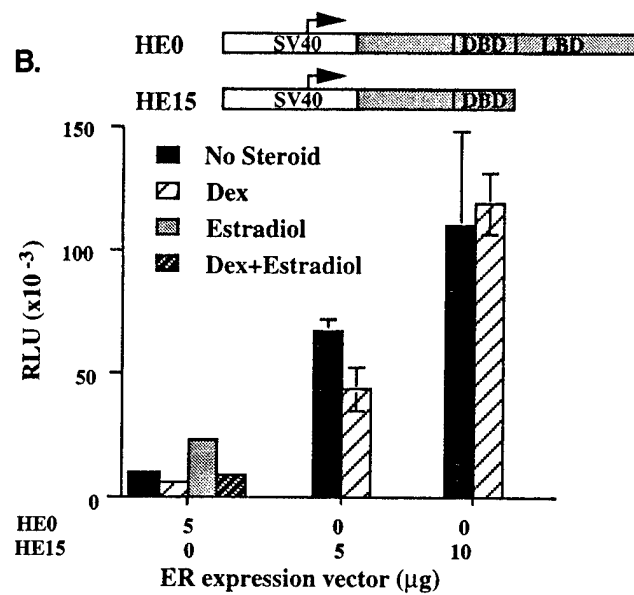
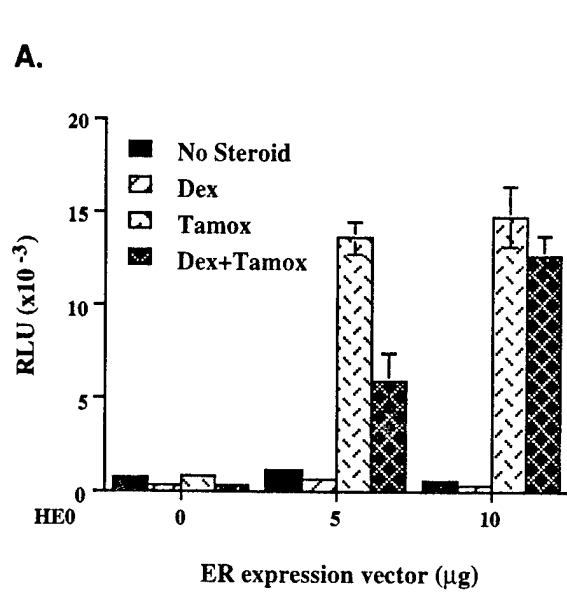


Figure 3

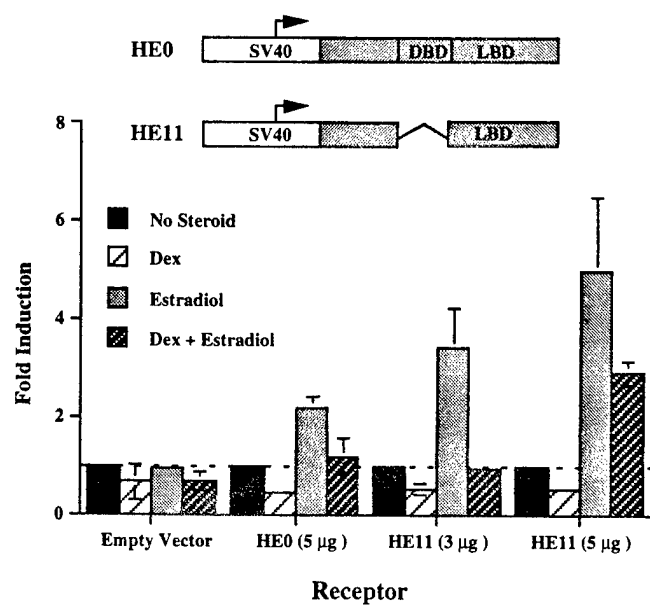


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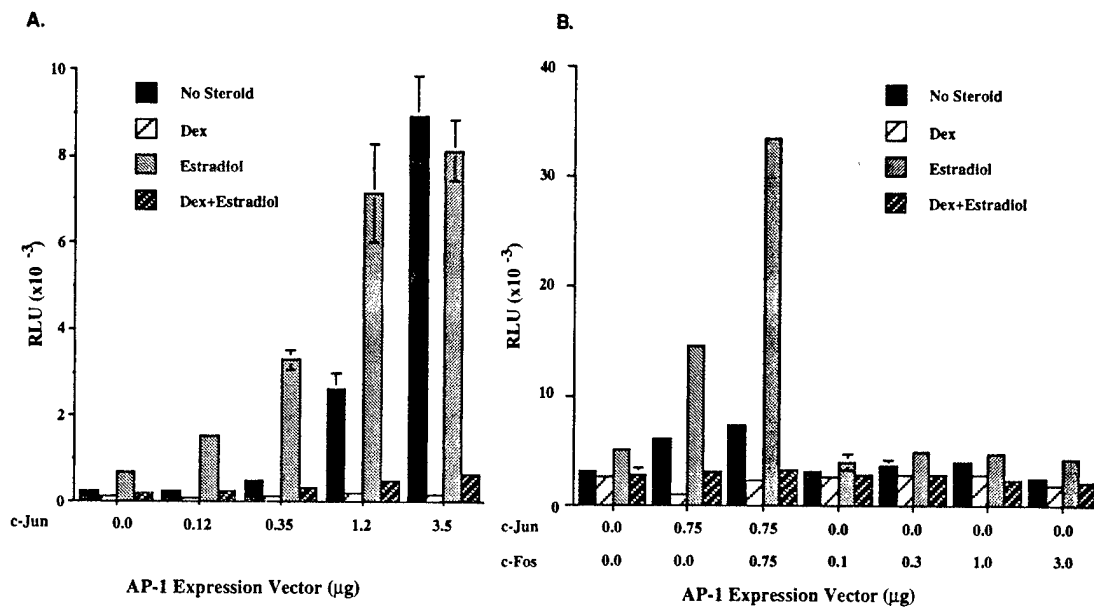


Figure 5

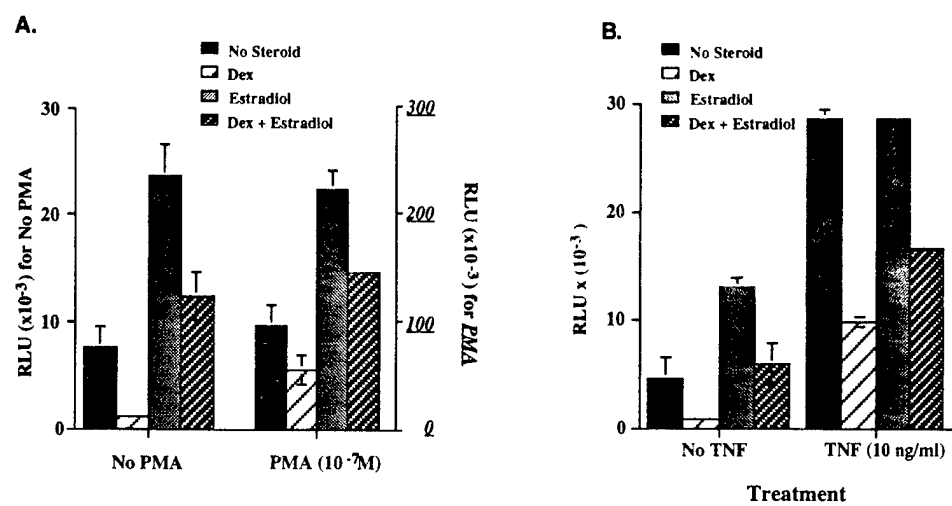


Figure 6

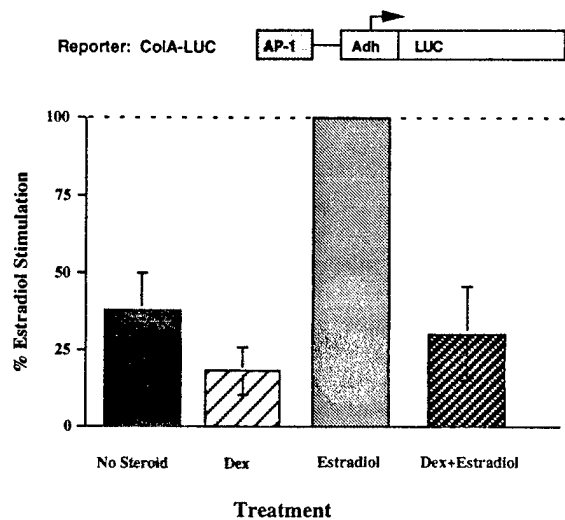


Figure 7

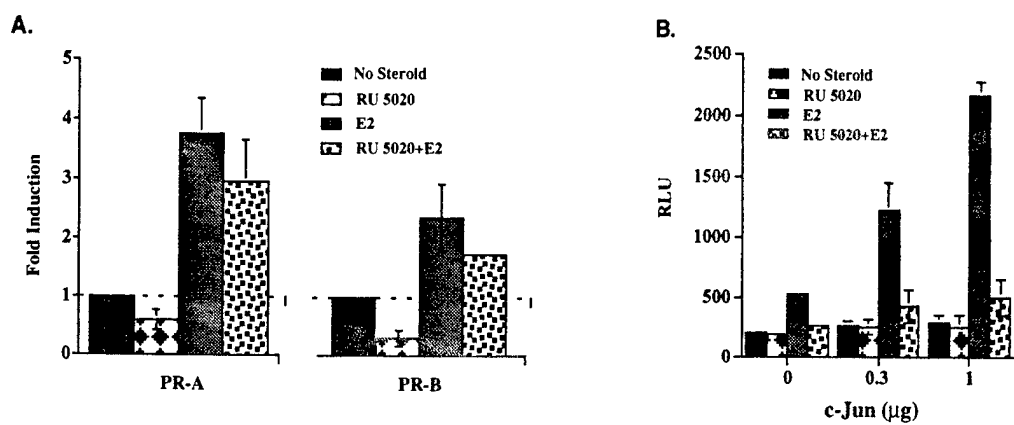


Figure 8